Discovery of Leonuri and therapeutical applications: From bench to bedside

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Abstract

Despite several advances in percutaneous coronary intervention and the discovery of new drugs, the incidence of myocardial infarction and deaths due to cardiovascular diseases (CVD) has not decreased markedly in China. The quality of life is affected seriously, which further results in great social and family burden. Many drugs, from the century-old aspirin to the newly FDA-approved Byvalson, have been proven to be effective in the treatment and prevention of CVD. As clinically reported, those life-saving drugs still have their side effects in regards to the narrow therapeutic indexes influenced by individual genetic variations. Herba Leonuri, also known as Chinese Motherwort, which are naturally present in plants and traditionally are used for the uterotonic action, postpartum blood stasis, breast pain as well as other gynecological disorders in China for thousands of years. Since the last two decades, our group has reported leonurine, a unique alkaloid found in Herba Leonuri, exhibits various bioactivities such as antioxidant, anti-apoptotic effects, free radical scavenging and anti-inflammatory effects, in addition to improving micro-circulation. These bioactivities are related to the underlying mechanisms of ischemic heart diseases and cardiac fibrosis. Pharmacological studies have proven leonurine to be effective in treating CVD in various ways, particularly ischemic heart diseases. Besides the cardio protective effects, which are similar in the central nervous system, more specifically, inhibited mitochondrial reactive oxygen species production together with the restored mitochondrial function and redox state were observed in middle cerebral artery occlusion rats by leonurine treatment, which strongly reveals its neuroprotective effects and carries a therapeutic potential for recovery and prevention of stroke. Based on their mode of action, we propose that leonurine can be developed as drugs to treat ischemic heart diseases. Taking advantage of the most recent findings in pharmacological research including the effects of low toxicity and good pharmacokinetics characteristics, leonurine has a very attractive prospect of clinical application. Our recent promising pharmacological results may be able to eradicate the barrier hindering its sale on market. In sum, from bench to bedside is no longer a long way for leonurine.

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Abbreviations: CVD, cardiovascular diseases; ROS, reactive oxygen species; MI, myocardial infarction; ECM, extracellular matrix; HIF, hypoxia-inducible factor; VEGF, vascular endothelial growth factor; NF-κB, nuclear factor-κB; DOX, doxorubicin; MDA, malondialdehyde; MMPs, metalloproteinases; AngII, angiotensinII; Nox, NADPH oxidase; AD, Alzheimer’s disease; Aβ, amyloid-β; CREB, camp-response element-binding protein; BDNF, neurotrophic factor; TrkB, tropomyosin-related kinase B; BBB, blood-brain barrier; BMECs, brain microvascular endothelial cells; tMCAO, transient middle cerebral artery occlusion; OGD/R, oxygen-glucose deprivation and reoxygenation; HDAC, Histone deacetylase; TJs, tight junctional proteins.

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1. Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide with an estimated 17.5 million deaths annually calculated from the World Health Organization (Khan, Marvel, Wang, and Martin, 2017). People's lifestyles such as alcoholism (Lin et al., 2017), smoking (Wang et al., 2017), obesity (Triggiani et al., 2017), hypertension (Wang et al., 2017), as well as environmental pollution (Feigin et al., 2016) and all those unhealthy factors contribute greatly to the occurrence and development of CVD. Moreover, the incidence of CVD will remain an upward trend in the next few decades (Weiwei et al., 2016). Since the thiazide diuretics used to treat essential hypertension in 1957, the development of anti-cardiovascular drugs has made great strides. The current clinical application of drugs for the treatment of cardiovascular disease is a wide range including calcium antagonists, anti-arrhythmic drugs, anti-chronic heart failure drugs, anti-angina drugs, anti-atherosclerosis drugs and antihypertensive diuretics or dehydratation drugs. But these drugs are not impeccable, regardless of a hundred years old drug aspirin or the combined drug Byvalson approved newly by FDA, due to the intricate side effects caused by the narrow therapeutic indexes and individual distinction of genotypes. For the past few years, pharmacology and pharmacological studies have made the clinical application of Chinese medicine more objective and scientific. As the thousands of years of gynecological herbal medicine inherited from the Compendium of Materia, herb leonuri also named motherwort is commonly used in Chinese herbal medicine for the treatment of postpartum blood stasis, menorrhagia and menoxenia. Leonurine is the main active compound of motherwort, and first time we had uncovered the cardiovascular protection of leonurine through the anti-apoptotic and anti-inflammatory effects in the past several decades. Furthermore, what excited us is its unique protective effect on central nervous system after stroke. Leonurine can inhibit mitochondrial reactive oxygen species (ROS) production and adenosine triphosphate biosynthesis in rat middle cerebral artery occlusion model. In consideration of its admirable cardiovascular treatment effects, we also innovatively optimized the synthesis route to produce large number of leonurine. As the saying goes, the Great Wall is not built on the day, in spite of the superiorities of leonurine in druggability such as low toxicity and good pharmacokinetics characteristics, the real potential mechanisms for treating cardiovascular disease remain to be tapped, the recent series of encouraging experimental results lend significant therapeutic promise as it translates from bench to bedside.

2. History

2.1. Herba Leonuri

Herba Leonuri, also named Chinese Motherwort or Siberian Motherwort, is native to China, central Europe, Scandinavia and Russia, but now it is also popular in Japan, Java, Malaysia and North America. Thousands of years ago, Leonurus japonicus has been recorded as “Top grade” in the oldest classical medicinal book Sheng Nong Ben Cao Jing (a bible of Chinese medicine) in China. In Ben Cao Gang Mu (another most famous masterpiece of Chinese medicine), it was considered to be non-toxic and harmless and used for treatment of vaginal bleeding, dystocia, retained fetal membranes, bruising, metrorrhagia, metrorrheasis, hemuresis and some other diseases, which has been circulating so far. Since 1990, it also has been listed in the Pharmacopoeia of the People's Republic of China, in which more than 15 kinds of traditional Chinese medicine prescription contain Leonurus japonicus as the main active component (Shang, Pan, Wang, He, and Li, 2014). Coincidentally, the description of Leonurus japonicus is also emerged in the seventh edition of the European Pharmacopoeia. Since modern times, western researchers seem to have reached an agreement with oriental scholars on the treatment effects of Leonurus japonicus on gynecological diseases. Besides, it is applied in nervous conditions as an aid in hyperthyroidism owing to its efficacy to remit the palpitations and tachycardia associated with hyperthyreosis (Wojyniak, Szymanski, and Matlawska, 2013).

2.2. Leonurine

In 1976, Yeung CH put forward that leonurine extracted from the leaves of Herba Leonuri was an uterotonic demonstrated in rat uterus in vitro. Results from this study suggested that leonurine is able to develop into a new and effective drug based on ethno-botanical experiment, which is the first scientific description about leonurine to be a drug (Kong et al., 1976). In the following year, Cheng KF further confirmed the structure of leonurine and its uterotropic effect in vitro (Yeung, Kong, Lay, and Cheng, 1977). In addition, Kong YC developed a synthetic procedure of leonurine and showed, the chemical synthesized leonurine had the same uterotropic effect compared to natural extraction (Cheng, Yip, Yeung, and Kong, 1979). After entering the 21st century, the number of researches about leonurine was blown up and most of which concerned the protective effects of leonurine on cerebral vascular diseases (Chen and Kwan, 2001; Liu, Xin, Hou, and Zhu, 2009; Loh et al., 2010; Zhang et al., 2017).

3. Active chemical compounds of traditional Chinese medicine Herba Leonuri

It has been reported that approximately 140 chemical components had been isolated and identified from Herba Leonuri including alkaloids, flavonoids, and diterpenes (Shang et al., 2014). In addition, Herba Leonuri also contains plentiful amounts of potassium and vitamins, all of which exert special beneficial effects in CVD and cerebral ischemia. Among these compounds, alkaloids are considered a major class of active ingredients of Herba Leonuri. The total amount of alkaloids ranges from 0.1% to 0.2%; higher alkaloids content is found in younger and more succulent plants (Liu, Pan, and Zhu, 2012b).

3.1. Alkaloids

Alkaloids are dominated in Herba Leonuri, the main four alkaloids have been identified: leonuridine, leonurine, leonurinine and stachydrine. To date, lots of reports have shown that the alkaloids exert beneficial effects in ischemic cardio-cerebrovascular diseases, fortunately, it also be verified by our recent work on leonurine showing the protective effects on ischemia both in vivo and in vitro via different mechanisms.

Leonurine, a unique alkaloid, possessing several pharmacological effects, such as an uterotonic action, antiplatelet aggregation and inhibition of vascular contractile responses (Wang, Zhang, and Ju, 2004), has received more attention than any other chemical substances in Herba
Leonuri (Zhu et al., 2004). Recently, leonurine displays the wide variety of biological activities, including antioxidant, anti-ischemic, anti-apoptotic, and anti-inflammatory effects in our group (Liu et al., 2009; Liu, Pan, Wang, Gong, and Zhu, 2012a; Liu, Xin, and Zhu, 2007). Surprisingly, leonurine can exert its cardioprotective effects via different mechanisms. For example, leonurine inhibits intracellular ROS and upregulates levels of phosphorylated Akt, leading to a further increase in the Bcl-2/Bax ratio and preservation of mitochondrial function, in turn, which attenuates caspase activation and reduces apoptosis. At the same time, leonurine upregulates hypoxia-inducible factor (HIF)-1 levels and triggers the expression of vascular endothelial growth factor (VEGF), which also contributes to cardioprotection. Furthermore, leonurine attenuates inflammatory responses by inhibiting intracellular ROS production and nuclear factor (NF)-κB activation in CVD.

Accumulating evidence demonstrates alkaloids of Herba Leonuri, especially leonurine attenuating cardiac dysfunction and inhibiting cardiovascular inflammatory responses, but the precise mechanisms are still to be clear.

3.2. Flavonoids

It has been reported that approximately 30 flavones from Herba Leonuri have been isolated and identified, such as rutin, kaempferol, queretin and so on. Rutin has multiple pharmacological actions, including antioxidant, cytoprotective, antiplatelet, antithrombic, vasoprotective and cardioprotective. Priya S et al. showed that rutin prevents against isoproterenol induced cardiac damage partly through the free radical scavenging, antioxidant and stabilizing lysosome membrane (Chen and Kwan, 2001). In addition, rutin elevates the Bcl-2/Bax ratio, as well as endoplasmic reticulum stress proteins to exert its anti-apoptotic effects in H9c2 cells (Kim et al., 2010). Flavonoids are widely found in fruits and vegetables and are believed to promote optimal health partly through enhancing cellular anti-oxidant capacity. It has been affirmed that flavonoids intake may uncover new strategies for the treatment of cardiovascular diseases.

3.3. Diterpenes

Previous review has demonstrated that dozens of diterpenes have been isolated and identified from Herba Leonuri and all are labdane diterpenoids (Shang et al., 2014). Due to their various bioactivities and toxicities, growing attention has devoted to those complex structure and intriguing chemistries. Marrubin, a diterpenoid found in Leonotis leonurus, largely contributes to the extract’s anticoagulant, antiplatelet and anti-inflammatory effects by inhibiting the binding of fibrinogen to glycoprotein (GP) IIb/IIIa receptor (Mononopf, Levendal, Daviss-Coleman, and Frost, 2011). A study conducted by Peng C et al. presented that the leonuketal, a Spiroketal Diterpenoid from Herba Leonuri exert significant vasorelaxant activity against KCl-induced contraction of rat aorta (Xiong et al., 2015). In a word, diterpenes with multidimensional biological effects has beneficial therapeutic properties.

4. The distinguished pharmacological activity and the diversified mechanisms of leonurine make it the prospect of developing new drug in CVD

Leonurine also named SCM-198 from our group, an active phenolic alkaloid only found in Herba Leonuri. It was originally reported to have outstanding uterine contraction and diuretic effects (Melendez-Camargo, Conteras-Leon, and Silva-Torres, 2014; Pang et al., 2001; Yeung et al., 1977). Surprisingly, leonurine exhibits an extensive variety of biological activities on CVD via different mechanisms (Fig. 1). Furthermore, the novel discoveries push us to extend the application of leonurine to the cerebrovascular diseases, where Chinese herbal medicine once again demonstrated its powerful effects with disparate pathway compared to the former. To sum up, all the gratifying pioneered results from our group convince us that leonurine as a traditional Chinese medicine is drug-able agent and we are on the right way from bench to bedside for leonurine (filed U.S. FDA and China FDA clinical trial license recently).

4.1. Cardiovascular protective effects of leonurine

4.1.1. Leonurine attenuates doxorubicin-induced apoptosis in H9c2 cells

Doxorubicin (DOX) is a frequently-used antineoplastic drug used to treat a variety of cancers including lymphomas, leukemias and solid tumors (Hortobagyi, 1997; Singal and Iliskovic, 1998; Young, Ozols, and Myers, 1981). However, the side effects that DOX-induced apoptosis in cardiomyocytes cause irreversible degenerative cardiomyopathy and heart failure, which limits its use in clinical practice (Singal and Iliskovic, 1998). Pre-treated with leonurine could attenuate DOX-induced cell apoptosis, reduce malondialdehyde (MDA) formation and intracellular Ca²⁺ overload, moderate the dissipation of mitochondrial membrane potential caused by DOX (Xin, Liu, and Zhu, 2009). Significant efforts have been made to cut down DOX-induced cardiotoxicity and strengthen its antineoplastic efficacy (O’Brien et al., 2004). More importantly, leonurine did not affect DOX’s anticancer effect in some solid carcinoma cell lines. Because of the cardioprotective effect against DOX-induced cardiotoxicity, thereby, leonurine is a valuable resource for combination with DOX.

4.1.2. Protective effects of leonurine in hypoxic neonatal rat cardiomyocytes and infarcted heart

Myocardial infarction (MI) remains the leading cause of death in the world. One of the most typical features in patients with MI is hypoxia, which is the cause of morphological and biochemical changes in cultured newborn cardiomyocytes (Tanaka et al., 1994). Programmed cell death or apoptosis is considered as a physiological counterpart of cell replication and is the vital cause to cardiomyocyte cell death when MI and heart failure happen (Narula et al., 1996; van den Hoff, van den Eijnde, Viragh, and Moorman, 2000). So, anti-apoptotic therapy is one of the effective strategies after ischemia/reperfusion-induced infarct expansion (Vakeva et al., 1998). Besides, ROS can be generated as a result of ischemic myocardium (Qin, He, Hai, Liang, and Liu, 2008). As a common mediator of apoptosis, oxidative stress is directly implicated in the initiation of apoptosis (Greenlund, Deckwerth, and JohnsonJR., 1995). In both physiological and pathophysiological condition, cytosolic Ca²⁺ plays a crucial role in controlling cardiac contraction and relaxation. Intracellular Ca²⁺ overload is one of the main factors in ischemic injury and leads to cellular injury and cell death (Carrozza Jr. et al., 1992; Mochizuki and MacLeod, 1997). Leonurine exerts cardioprotective effects by upregulating the expression of the anti-apoptosis genes Bcl-2 and Bcl-xl and downregulating the expression of the pro-apoptosis genes Fas and Bax following hypoxia, at the same time, it seems to work by augmenting the activity of the anti-oxidant enzymes such as SOD and CAT, as well as inhibiting lipid peroxidation in hypoxia cardiomyocytes (Sun et al., 2005), furthermore, leonurine could suppress the cytosolic Ca²⁺ overload, which was likely to block L-type Ca²⁺ channel or change the antiapoptosis gene expression related to Ca²⁺ homeostasis (Liu, Chen, Pan, Silva, and Zhu, 2009). In vivo, leonurine could remarkably decrease infarct size in MI rats (Liu et al., 2009). These studies will tap the mechanical basis for the development of leonurine in clinical application for patients with cardiovascular diseases.

4.1.3. Leonurine improves cardiac recovery in rat during chronic infarction

In the cardiovascular system, as a critical regulator, Akt signaling pathway takes part in the progress of hypertrophy, angiogenesis and apoptosis (Bhuiyan and Fukunaga, 2009). Demonstrated results suggest the activation of this pathway can promote myocytes survival in the MI (Fujio, Nguyen, Wencker, Kitis, and Walsh, 2000). Together with the anti-apoptotic effect, activating Akt preserved cardiomyocyte function both in vivo and in vitro, while inhibiting Akt activity aggravated...
Leonurine can exert various cardioprotective effects including anti-apoptosis, anti-atherosclerosis, anti-fibrosis and shrinking the myocardial infarct size. It is with extensive mechanisms to exert its vigorous protective effects. For example, leonurine inhibits intracellular reactive oxygen species (ROS) accumulation directly or through blocking the upstream inducer Nox4 and hypoxia-inducible factor (HIF)-1 level, leading to a further elevated the ratio of Bcl-2/Bax and reduced inflammatory factors. Besides, leonurine is found to be a potential inhibitor of L-type calcium channel, preserving the myocardial cell against apoptosis.

In summary, leonurine administration not only attenuated the inflammatory processes but also inhibited the cardiac fibrosis partly through blocking the activation of Nox4 and NF-κB. ROS act as second messenger and stimulate release of inflammatory mediators, thereby leading to cardiac fibroblast activation and myocardial fibrosis (Bedard and Krause, 2007). As expected, leonurine prominently decreased ROS formation accompanied by reduced pro-inflammatory factors and collagen synthesis in cardiac fibroblasts. In conclusion, the inhibiting myocardial fibrosis by leonurine is associated with suppression of a Nox4-ROS-NF-κB pathway (Liu et al., 2013).

4.1.5. Leonurine might be a potential agent for the treatment of atherosclerosis

Atherosclerosis, a progressive chronic inflammatory vascular disorder, is one of the most common diseases affecting human’s health, with high morbidity, disability and mortality (Ross, 1999). Recent researches have revealed that inflammation and oxidative stress are cooperatively involved in the pathological process of atherosclerosis (Libby, 2006). Recruitment of monocytes to the endothelium is a main feature in the early stage of atherosclerosis. Since ROS could induce the inflammatory process via various pathways, increased oxidative stress appears to play an important role in genesis and development of atherosclerosis (Catapano, Maggi, and Tragni, 2000). Long-term administration of leonurine could reduce low density lipoprotein cholesterol, total cholesterol and triglyceride, and retard the progression of early atherosclerotic lesions to advanced plaques in atherosclerotic rabbits. These effects of Nox4 is a major source of ROS in heart and plays a vital role in the activation of cardiac fibroblasts and remodeling (Bedard and Krause, 2007; Kuroda et al., 2010). Therefore, restraining the activation of Nox4 is a priority objective in the pathological process of unfavorable cardiac remodeling. The previous results reported that leonurine inhibited Nox4 expression, ROS production and ERK1/2 activation induced by Ang II, which further prevented MMP-2/9 as well as the type I and III collagen expression in cardiac fibroblasts (Arimura et al., 2001). In post-MI rats, leonurine administration not only attenuated the inflammatory processes but also inhibited the cardiac fibrosis partly through blocking the activation of Nox4 and NF-κB. ROS act as second messenger and stimulate release of inflammatory mediators, thereby leading to cardiac fibroblast activation and myocardial fibrosis (Bedard and Krause, 2007). As expected, leonurine prominently decreased ROS formation accompanied by reduced pro-inflammatory factors and collagen synthesis in cardiac fibroblasts. In conclusion, the inhibiting myocardial fibrosis by leonurine is associated with suppression of a Nox4-ROS-NF-κB pathway (Liu et al., 2013).

4.1.4. Leonurine attenuates myocardial fibrotic response via inhibition of NADPH oxidase 4

Cardiac inflammation and extracellular matrix (ECM) accumulation are the significant characteristics in the process of heart failure (Pfeffer and Braunwald, 1990). Cardiac fibroblasts play a vital role in the maintenance of ECM in the normal heart and as mediators of myocardial remodeling in failing heart (Chen, Chen, Li, Zhang, and Mehta, 2004). When cardiac fibroblasts are induced by angiotensinII (AngII), cardiac fibroblasts become extremely proliferative and invasive to remodel the interstitium via increasing secretion of ECM-degrading matrix metalloproteinases (MMPs) and augmenting the collagen accumulation (Sun, 2009), that cause the progressive cardiac remodeling process during MI (Peterson et al., 2001). Therefore, it is popular to intervening cardiomyocyte dysfunction. It is also admitted that induction of neovascularization is expected to be a valid approach to attenuate the pathophysiological changes in MI. HIF-1α mediated by Akt has been proven to enhance angiogenesis in MI mice (Matsunaga et al., 2009). In summary, Akt activating can benefit cardiac contractility and neovascularization in MI disease. Leonurine dramatically improved myocardial function as demonstrated by the decreased left ventricle end-diastolic pressure and the increased +dp/dt. Meanwhile, leonurine elevated the phosphorylation of Akt. Intriguing, leonurine not only increased the expression of HIF-1α but also the survivin and VEGF. The results indicated that leonurine improved myocardial function regulated by activating the PI3K/Akt signaling pathway in chronic infarction rats. Speculatively, angiogenic mechanisms may partially participate in the protective effects of leonurine, and more research is needed (Liu, Pan, Gong, and Zhu, 2010).
were associated with the attenuation of oxidative stress and prevention of chronic inflammation, including decreased inflammatory factors, such as TNF-α, IL-6 and VCAM-1 (Zhang et al., 2012). Coincidentally, another work from our lab also drew the analogous conclusions about the anti-inflammatory effects of leonurine on human umbilical vein endothelial cells by inhibition of NF-κB (Liu et al., 2012a). Taken together, leonurine exerts an atheroprotective effect on the progressive of hypercholesterolemic rabbits, which provides the basis for the application of leonurine as an anti-atherosclerotic drug.

4.2. The effects of leonurine on neurodegenerative disorders and ischemic stroke

4.2.1. Leonurine ameliorates cognitive deficits, promotes neuronal survival in Aβ/PP/PS1 mice

Alzheimer’s disease (AD), the most prominent neurodegenerative disorder, is a chronic progressive disease characterized by cerebral deposition of senile plaques constituted with amyloid-β (Aβ) peptides, neurofibrillary tangles, neuronal loss and neuroinflammation (Huang and Mucke, 2012). Various therapeutic strategies have been proposed and so much effort has been devoted to the drug discoveries, but it is still an incurable disease (Raina et al., 2008). Currently, the most notable hypothesis of AD is the Aβ cascade model making it a promising drug target for treating AD. Many studies also noted that cognitive deficits could be alleviated without altering Aβ burden, but the neurotoxicity of Aβ is identified and widely accepted (Doddart et al., 2002). Pioneered evidence showed that Aβ could inhibit Camp-response element-binding protein (CREB) phosphorylation, causing reduced long-term potentiation (LTP) to alter hippocampal-dependent synaptic plasticity (Saura and Valero, 2011). It has reported that CREB is required for the formation of long-term memory and the level of CREB phosphorylation is decreased in the model of AD (Vitollo et al., 2002). As the one of the target genes of CREB, brain-driven neurotrophic factor (BDNF) can active tropomyosin-related kinase B (TrkB), which play critical roles in neuronal survival and synoptic plasticity. Hence, enhancing CREB/BDNF/TrkB signaling pharmacologically or genetically is considered feasible for AD treatment (Meng, He, and Xing, 2013). Leonurine has been studied for the treatment of ischemia stroke, AD and Parkinson’s diseases mainly through the inhibition of oxidative stress, mitochondrial protection and alleviation of neuroinflammation (Hong, Shi, Zhu, Wu, and Zhu, 2014a; Loh et al., 2010). Extraordinarily, leonurine exhibited the potential neuroprotective effects in Aβ3 protein presenilin-1 (Aβ1/PP/PS1) double-transgenic mice, and the mechanisms associated with CREB/BDNF/TrkB pathway. To our surprise, leonurine could improve cognitive function and memory with no change in Aβ burden in Aβ1/PP/PS1 transgenic mice fed with leonurine for three months, which was extreme in this transgenic model. Leonurine also could promote BDNF/TrkB neurotrophic signaling partly through regulating the upstream PKA-CREB pathway. Leonurine had no ability to reduce Aβ1/PP expression or brain Aβ burden, but it exerted considerable neuroprotective and cognition-improving effects. Meanwhile, leonurine could inhibit microglial overactivation in Aβ1/PP/PS1 mouse brain possibly due to the anti-neuroinflammatory properties, which was in agreement with study in Aβ1 injected rat model (Hong, Shi, Zhu, Wu, and Zhu, 2014b). Taken collectively, leonurine alleviated cognitive deficits in Aβ1/PP/PS1 mice through inhibiting microglial overactivation, activating CREB/BDNF/TrkB signaling, indicating that leonurine could become a promising candidate drug for AD therapy (Fig. 2).

4.2.2. Novel therapeutic effects of leonurine on ischemic stroke: new mechanisms of BBB integrity

Stroke is another leading cause of morbidity and mortality in the world, owing to its limited therapeutic time window and fewer emergency medicines. Meanwhile, the secondary damage caused by reperfusion will cause worse outcomes including blood-brain barrier (BBB) breakdown, inflammation and post-ischemic neuronal injury (Radermacher et al., 2013). When ischemic and hemorrhagic stroke, multiple sclerosis and brain tumor occurs, BBB is broken associated with edema formation, inflammatory cascade and ultimately serious outcomes (Khan et al., 2012). The brain microvascular endothelium cells (BMECs) are considered as the basis of BBB, which establish a barrier restricting diffusion of blood-born solutes. The tight junctional proteins (TJs) containing occludin, claudins junctional adhesion molecules (JAM) as well as cytoplasm accessory zonula occluden (ZO) protein and caveolin-1 are responsible for the integrity of BBB in BMECs (Mark and Davis, 2002). Studies have demonstrated that following subjected to transient middle cerebral artery occlusion (tMCAO) or oxygen-glucose deprivation and reoxygenation (OGD/R), loss of BMECs evoked stress fibre formation and TJ redistribution result in shrunk cell morphology, enhancing permeability of BBB, but which could be reversed by preventing the BBB breakdown (Shi et al., 2016). Leonurine significantly reduced infarct volume and ameliorated neurological deficits in the tMCAO model, as well as improved TJs level in vitro the OGD/R model using BMECs. Treatment with leonurine at 0.5 h post-surgery exerted better therapeutic effect on infarct area and neurological deficit score than the classical drug Edaravone in the tMCAO animals. Meanwhile, leonurine remarkably decreased the BBB permeability and declined the brain edema and water content in the bilateral hemisphere. Following the initial phase of stroke, the second phase comes 24–48 h later with the vast expression and activation of MMPs, which then infested TJs to disrupt the integrity of BBB, however, it was restrained by leonurine administration, particularly the expression of MMP9. Histone deacetylases (HDACs) control a wide array of biological processes. Unexpectedly, as the downstream of HDAC4, NOX4 and MMP-9 were downregulated indirectly by leonurine to improve TJs level and therefore protect against BBB breakdown. In sum, it was the first time shown that HDAC4 was involved in regulating BBB integrity and leonurine had the protective effects against BBB leakage via increasing the expression of HDAC4 (Fig. 2).

5. Preclinical pharmaceutical research of leonurine

5.1. The chemical synthesis of leonurine

Because of the low content in Chinese Motherwort and variety of other ingredients acting as impurities, so chemical synthesis of leonurine is need for mass production. So far, there are several research groups have made a preliminary exploration of its synthesis and the initial synthetic route has been open up (Cheng et al., 1979; Shao, Mo, Zhu, and Hu, 1984). The general process is based on succinic acid as the starting material to prepare the intermediate product leonuriamine through Gabriel reaction, then react with S-methyl isothioura sulfate to generate the leonurine. The above synthetic route is simple and the raw materials are more expensive, but the low final yield, pollution-carrying reagents and the intractable intermediate products should be paid attention to as the drawbacks. On this basis, the optimized the synthetic path avoiding the Gabriel reaction was explored. As demonstrated in the Scheme 1 below, leonurine was prepared from S-methyl-isothioura, 4-amino-1-butanol and caryophylic acid. Compound 4 and compound 6 were the key intermediates in the strategy contributed to their high solubility in dichloromethane, which allowed the synthesis of leonurine to be performed even under mild conditions. In brief, S-methyl-isothioura condensed with 4-amino-1-butanol to obtain intermediate 3, which was further protected using Boc anhydride to afford key intermediate 4; the Phenolic hydroxyl group, which belongs to Caryophylic acid, was protected by acetic anhydride to gain another key intermediate 5. The resulting intermediate 6 was further condensed with intermediate 4 to afford intermediate 7, then the intermediate 7 was deprotectteunder basic condition to acquire intermediate 8, and finally, the Boc groups in 8 were deprotected under acidic condition to eventually get leonurine. The optimized synthesis suited for large-scale
production with all reactions involved being under mild conditions and high yields (Luo, Gu, and Zhu, 2012).

5.2. Pharmacokinetics of leonurine

To date, limited studies on the pharmacokinetics of leonurine have been reported. Recently, our research group has developed a sensitive and reliable liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) quantitative detection method and has used it to investigate the preclinical pharmacokinetic profiles of leonurine (Zhu et al., 2012). It has been found that the plasma concentration-time curves of leonurine fits the two-compartment open model both in rats and dogs. The elimination half-lives of leonurine ($t_{1/2}$ of 2.2–3.5 h in rats and of 0.9–2.3 h in Beagle dogs) after intravenous (i.v.) injection or intragastric (i.g.) administration indicate that leonurine may be quickly eliminated. The elimination of leonurine in rats after i.g. administration at dose of 15, 30, 60 mg/kg fits the linear kinetics characteristics which demonstrate similar elimination half-life ($t_{1/2}$ of 2.8–3.5 h), systemic clearance (CL of 70–125 L/kg/h), and mean residence time (MRT of 4.1–5.2 h), and the absorption rates at three dose levels are also similar based on the fact that there is no significant difference in the time to reach peak concentration ($T_{\text{max}}$ of 0.75–1.5 h) and mean absorption time ($T_{\text{MAT}}$ of 2.3–3.4 h). The absolute oral bioavailability of leonurine is very low (about 4.2% in rats and 7.0% in dogs), which may be explained by extensive intestinal first-pass elimination (gastrointestinal extraction ratio of about 90% in rats) (Fig. 3).

Fig. 2. The schematic diagram of neuroprotective effects of leonurine. Eminently, leonurine could keep the integrity of blood brain barrier preventing after stroke. The elevated the expression of HDAC4 induced by leonurine could inhibit the transcription of Nox4 in microvascular endothelial cell. The inhibition of Nox4 lowered the expression of MMP9, which finally achieve the purpose of neuroprotective effects. In the condition of Alzheimer's disease (AD), leonurine ameliorated cognitive deficits, promoted neuronal survival without affecting Aβ burden via CREB/BDNF/TrkB signaling, which could be repressed by adding the inhibitor of PKA. Meanwhile, the anti-inflammatory effects of leonurine in astrocyte also possibly contributed to neuroprotective effects.

Scheme 1. Reagents and conditions: (i) H2O, TEA, r.t.; (ii) di-tert butyl dicarbonate and Na2CO3 in THF, r.t.; (iii) TEA, Ac2O in DCM, r.t.; (iv) DIC, DPTS, r.t.; (v) sodium methoxide, 4 °C r.t.; (vi) TFA.
The results of tissue distribution tests in rats show that leonurine is widely distributed into various tissues. The highest concentration of leonurine is detected in small intestine, followed by liver and kidney. In addition, a moderate amount of leonurine is detected in heart, lung, spleen, and pancreas, suggesting that these organs may be the target organs of drug action. However, limited distribution to the brain indicates that leonurine is difficult to cross the BBB. Interestingly, we detect a moderate amount of leonurine in cerebrospinal fluid in cerebral ischemic model rats, which may provide the basis for neuroprotection of leonurine. The plasma protein binding ratios of leonurine in plasma of rats, dogs, and humans show similar values at three concentration levels (50, 100, 200 μg/L) in one species but significant differences in different species (76–78% in rats, 37–40% in dogs, and 17–25% in humans), which indicates that the binding of leonurine to the plasma protein is linear within the concentration ranges tested, and the affinity to plasma protein may have species difference. The low to moderate ratios of plasma protein binding (less than 80%) suggests the low risk of the adverse drug reaction induced by drug-drug interaction on plasma protein binding ratio in the clinical practice.

The investigations on the metabolism and excretion of leonurine in rats have attracted a lot of interest. The excretion tests show that 0.5% and 17% of the total dose given is recovered in urine and feces, respectively. After β-glucuronidase treatment, the recovery of parent drug in urine is increased to 12.5%, while the recovery of parent drug in feces has no change. The results suggest that leonurine may suffer from extensive metabolism and glucuronidation may involve in the biotransformation. In metabolism studies, at least three metabolites including two phase II metabolites, glucuronide (M1) and sulfate conjugate (M2), and one phase I metabolite, demethylated leonurine (M3) were identified in rat in vivo samples after oral dosing (Fig. 4). Among the three metabolites, M1 is the absolutely predominant one, and it has been reported that the intensity of M1 is far higher (approximately 20-fold) than parent drug in the pooled plasma samples after oral dosing. Meanwhile, in intestinal tract metabolism test, M1 is also the leading metabolite. Then the metabolite M1 is characterized as leonurine-O-glucuronide by NMR spectroscopy (Zhu et al., 2014). Also, two primary metabolites, leonurine-O-glucuronide and demethylated leonurine, have been identified in pooled human liver microsomes and leonurine-O-glucuronide is the predominant one. The UDP-glucuronyl transferases (UGT) isoform UGT1A1 is the principal enzyme responsible for leonurine glucuronidation in human liver and intestine microsomes (Tan et al., 2014). These results showed the oral administered parent leonurine may undergo intensive first-pass metabolism (primary in intestinal tract and secondary in liver) to form the lead metabolite leonurine-O-glucuronide, which results in the high level of leonurine-O-glucuronide in vivo and the low systemic bioavailability. Interestingly, leonurine-O-glucuronide may have potential cardioprotective effect and its potency is similar to leonurine, which may explain why leonurine still exerts good pharmacological effect with such low bioavailability after oral dosing. The absorption, distribution, metabolism, and excretion profiles of leonurine in rats after i.g. administration are summarized in the following sketch (Fig. 3).

Leonurine is able to inhibit the activity of CYP1A2 in human microsomes with K_\text{d,0} value of 80 μM, but has no inhibition on other CYP450 isoforms, which suggests that drug-drug interaction induced by drug-drug interaction on plasma protein binding ratio in the clinical practice.
interactions may occur when leonurine is in combination with the substrate of CYP1A2 in clinical therapy.

6. Insights and prospective of leonurine

Statin therapy is the most efficient and fashionable way for the patients with CVD or the public to prevent the heart attacks and strokes. It is well known that HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase catalyzes the committed step in cholesterol biosynthesis. As the specific inhibitors of HMGR, statins effectively lower serum cholesterol levels and are widely prescribed in treating hypercholesterolemia (Istvan and Deisenhofer, 2001). But like all other medications, they have potential for adverse effects and the first and the most reported problem is on muscle. Other adverse effects such as cognitive loss, neuropathy, pancreatic and hepatic dysfunction and sexual dysfunction have released from randomized controlled trials worldwide (Golomb and Evans, 2008). As to leonurine, there were no prominent adverse effects have being exposed in our toxicity tests on multiple experimental animals, which make it an additional choice for the patients especially those who are sensitive to statins. On the other hand, the consumption of statins is much lower in low-income and middle-income countries compared in developed countries and even 80.2% patients with coronary heart disease receive no drugs in low-income countries (Yusuf et al., 2011). The high cost of statins owing to its complex synthesis procedure mainly contributes to the limited application in low-income countries, however, the leonurine could be more affordable for the people. It is high cost performance using leonurine to prevent CVD as it could be applied in much more indications. Relative to the explicit mechanism of statins, it is still mysterious for us about its rationales; as far as we known, the vascular microenviroment and homeostasis should be involved in the process. In a word, incomputable effort should be paid to uncover the hidden mechanisms of leonurine treating CVD for an extended period.

7. Summary and conclusion

Herein, we comprehensively sketched the existing knowledge on Herba Leonuri, including its chemical constituents, traditional uses, biochemical and pharmacological studies, particularly the overriding alkaloid leonurine, also named SCM-198 in our lab. Recently, leonurine has been proven to have various pharmacological activities, such as antioxidative, cardiovascular and cerebrovascular effects, neuroprotective, anti-inflammatory, antifibrotic, and anti-infection activities. Despite showing good pharmacological or therapeutic effects, there is still a need for more precise researches to uncover the latent biological target and their mechanisms of action where possible. At the same time, what cannot be ignored is that very limited evidence from researches on the side effects of leonurine is demonstrated, especially using leonurine in combination with other drugs. In the past decades, great efforts have been made from our lab to make it druggable, from the basic optimized chemical synthesis, the pharmacodynamics evaluation both in vivo and in vitro and the all-round toxicology studies to the final dosage form examination. All of which is going to pave the way for successful entry into the drug counter, as a neoteric efficient drug available for patients with cardiovascular or cerebrovascular diseases. To sum up, from bench to bedside is no longer a long way for leonurine based on our recent promising pharmacological results.

Conflict of interest

The authors have no conflict of interest declare.

Fig. 4. The MS² spectra and fragmentation pattern of three metabolites. Metabolite M1 was eluted at the retention time of 4.9 min, which is 176 Da more than that of parent. M1 was glucuronide metabolite of leonurine. M2 was observed at the retention time of 6.9 min, which is 80 Da more than that of parent. M2 was sulfate conjugate of leonurine. As to M3, the retention time was 7.2 min, it is 14 Da less than that of parent, which indicated that one of the two methoxy group in parent is demethylated.
References


